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Short Communication

Activity-dependent Energy Metabolism in Rat Posterior Pituitary Primarily Reflects Sodium Pump Activity

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Recent studies suggest (Schwartz et al., 1979) that local cerebral glucose utilization, measured with the deoxyglucose technique (Sokoloff et al., 1977), correlates most closely with electrical activity in the neuropil in general and synaptic terminals in particular. Presumably, increased glucose utilization associated with increased impulse activity in nervous tissue is, as is oxygen consumption (Ritchie, 1967; Greengard and Ritchie, 1971; DeWeer, 1975), principally due to enhanced activity of the sodium pump. If the increased energy metabolism during impulse activity is used mainly for reconstitution of electrochemical gradients, then it is to be expected that cellular components with larger surface-to-volume ratios will have larger energy demands (Ritchie, 1967; Greengard and Ritchie, 1971; DeWeer, 1975) and, thus, greater rates of glucose utilization. It would be of value for the interpretation of studies that employ the autoradiographic deoxyglucose method to identify the cellular elements in which neural activity and energy metabolism are most closely linked. We have, therefore, studied an *in vitro* preparation of rat posterior pituitary, which represents a relatively enriched population of axon terminals (Nordmann, 1977) and may serve as a model for synaptic endings in the brain. Because the pituitary is a neurosecretory organ, we have also studied the influence of the secretory process in this system on energy metabolism. As an index of glucose utilization, we have measured the rate at which [^{14}C]deoxyglucose is phosphorylated by hexokinase and trapped in the tissue incubated *in vitro*. This is the *in vitro* equivalent of the deoxyglucose method in which trapped [^{14}C]deoxyglucose-6-phosphate is visualized and measured autoradiographically (Sokoloff et al., 1977).

MATERIALS AND METHODS

Male Sprague-Dawley rats (180–250 g) were decapitated, and the pituitary glands were removed rapidly and placed in balanced salt solution (BSS) consisting of 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (adjusted to pH 7.4 with NaOH), 130 mM-NaCl, 4.5 mM-KCl, 1.8 mM-$CaCl_2$, 0.9 mM-$MgSO_4$, and 11.1 mM-glucose. The final osmolarity of the solution was 290 mosM. One hundred percent oxygen was continuously bubbled through the BSS. The posterior pituitaries were gently teased from the anterior lobes. Posterior pituitaries from three to five rats (approximately 3–5 mg wet weight of tissue) were combined in a small sack made of 200-μm mesh Nytex. Following a 15-min equilibration at 37°C in the BSS, the tissue was transferred to a vial containing 13.6 μCi/ml of 2-deoxy-D-[1-^{14}C]glucose (54.4 mCi/mmol; New England Nuclear Corp., Boston, Massachusetts) in BSS. Pharmacological agents were included in some experiments as described below. In experiments carried out in the absence of calcium, the preincubation medium was also calcium-free. In experiments in which the tissue was stimulated electrically, the Nytex bag was placed between two platinum grid electrodes. After 5 min in [^{14}C]deoxyglucose-containing medium, the tissue was stimulated with 1-V rectangular pulses at 10 Hz for 10 min; the pulses were 0.4 msec in duration. In all of the experiments the tissue was incubated for a total of 15 min in the medium containing the [^{14}C]deoxyglucose. Experiments were terminated by washing the tissue for 50 min in five successive changes of BSS in order to remove any free deoxyglucose.

The tissue was digested in 1 M-NaOH and portions were taken for protein determination (Lowry et al., 1951) and for measurement of ^{14}C by liquid scintillation counting. The results are expressed as c.p.m. of [^{14}C]deoxyglucose/100 μg protein/15 min. In a separate experiment, the identity of the labeled product was determined by thin-layer chromatography. Eighty-eight percent of the radioactivity was in the form of [^{14}C]deoxyglucose-6-phosphate.

The results have been analyzed with Dunnett's statistical test for multiple comparisons with a single control group (Dunnett, 1955; 1964).

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Abbreviations used: BSS, Balanced salt solution; mosM, Milliosmolar; Hz, Cycles per second.

TABLE 1. *Effects of repetitive electrical stimulation on [^{14}C]deoxyglucose uptake in posterior pituitary*

Condition	[^{14}C]Deoxyglucose uptake (c.p.m./100 μg protein/15 min)	Statistical significance
Controls (4)	988 ± 19	
Stimulated at 10 Hz (4)	1272 ± 57	$p < 0.01$
Stimulated at 10 Hz + ouabain (4)	1018 ± 51	n.s.

The values represent the means ± S.E.M. of the results obtained in the number of experiments, indicated in parentheses.

RESULTS AND DISCUSSION

Electrical stimulation of the posterior pituitary *in vitro* at 10 Hz produced a 29% increase in [^{14}C]deoxyglucose-6-phosphate accumulation in the tissue above control values (Table 1). In the presence of 10μM-ouabain (an inhibitor of the Na^+-K^+-ATPase and sodium pump), however, electrical stimulation of the tissue produced no significant change in [^{14}C]deoxyglucose-6-phosphate accumulation from control values (Table 1). Inasmuch as ouabain does not inhibit either spike activity or hormone release in the neurohypophysis (Dicker, 1966), these data strongly indicate that activation of the ouabain-sensitive sodium pump is the critical event coupling energy metabolism to electrical activity.

Additional evidence was obtained in experiments with veratridine, an alkaloid that activates the sodium conductance mechanism in the membrane in association with spike activity. In the posterior pituitary, veratridine depolarizes the nerve terminals, produces an influx of sodium ions, and, consequently, causes a large release of hormones (Dyball and Nordmann, 1977). The results in Table 2 (A) show that in the presence of 60 μM-veratridine, deoxyglucose-6-phosphate formation was significantly increased over control values by an average of 37%. The addition of 6 μM-tetrodotoxin, a neurotoxin which blocks the activation of sodium channels, prevented the veratridine effect on deoxyglucose phosphorylation. The effect of veratridine appeared, therefore, to be mediated by its effects on the sodium permeability of cell membranes. Furthermore, as in the experiments with electrical stimulation, inhibition of the sodium pump by ouabain also prevented the veratridine stimulation of deoxyglucose phosphorylation [Table 2 (A)].

A major function of the terminals in the posterior pituitary is neurosecretion. In order to examine the contribution of the process of neurosecretion to energy consumption we measured the effect of veratridine on deoxyglucose phosphorylation in posterior pituitaries incubated in the absence of calcium and a raised magnesium concentration [Table 2 (B)]. Under these conditions neurosecretion is inhibited, but electrical activity is not (Dyball and Nordmann, 1977). In spite of the absence of neurosecretion, veratridine continued to cause a significant, 47% increase in deoxyglucose phosphorylation above the calcium-free control values. The contribution of neurosecretion to the energy utilization of these terminals appears, therefore, to be negligible in comparison with that of sodium-pump activity.

The results of the present studies suggest that the major energy-consuming function of activated nervous tissue is ion pumping by the sodium pump. Glucose utilization traced by deoxyglucose phosphorylation was increased in the posterior pituitary by electrical stimulation and by

TABLE 2. *Influence of sodium-pump activity and neurosecretion on [^{14}C]deoxyglucose uptake in posterior pituitary*

Condition	[^{14}C]Deoxyglucose uptake (c.p.m./100 μg protein/15 min)	Statistical significance
A. Dependence on activation of sodium-pump activity		
Controls (14)	1381 ± 50	
Veratridine (14)	1891 ± 85	$p < 0.001$
Tetrodotoxin (9)	1209 ± 84	n.s.
Tetrodotoxin + veratridine (8)	1551 ± 72	n.s.
Ouabain (4)	1318 ± 57	n.s.
Ouabain + veratridine (4)	1218 ± 120	n.s.
B. Independence from activation of neurosecretion		
Controls Ca^{2+}-free medium (6)	1142 ± 38	
Veratridine in Ca^{2+}-free medium	1681 ± 78	$p < 0.001$[a]

The values represent the means ± S.E.M. of the results obtained in the number of experiments, indicated in parentheses.

[a] The statistical significance of the difference between these two groups was calculated by Student's *t*-test.

veratridine, both of which produce an activation of the sodium conductance mechanism, with a consequent influx of sodium. With both modes of stimulation the increase in glucose utilization was prevented by ouabain, which blocks the sodium pump. Both electrical stimulation and veratridine should have activated neurosecretion, the primary function of the terminals in the posterior pituitary. If the secretory process, however, made a substantial contribution to the energy consumption of an activated nerve terminal, then the ouabain should not have blocked the effects of electrical stimulation and veratridine on glucose utilization. Furthermore, the veratridine stimulation of glucose utilization was unimpaired by incubation conditions that blocked neurosecretion. Therefore, we can find no evidence for a significant contribution of neurosecretion to the consumption of energy in nerve terminals.

Our results also show that the rate of glucose consumption by the nerve terminals of the posterior pituitary at rest is not affected by ouabain in the medium. Thus, relatively little energy metabolism at rest appears to be used for the maintenance of ionic gradients by the ouabain-sensitive sodium pump. The resting energy metabolism is probably devoted to other cellular processes, e.g., cell maintenance and repair, axoplasmic transport, and—in neuronal perikarya—protein synthesis. The proportions of total resting energy metabolism associated with each of these processes remain to be determined.

These results obtained with deoxyglucose uptake confirm and extend the results of other studies in which oxygen consumption was measured (Ritchie, 1967; Greengard and Ritchie, 1971; DeWeer, 1975). Increases in energy metabolism with stimulation are a function of the increased activity of the sodium pump which is activated by the influx of sodium ions and efflux of potassium ions accompanying a depolarizing stimulus. The increment in energy utilization accompanying membrane depolarization is, in turn, a function of the surface-to-volume ratio of the tissue (Ritchie, 1967; Greengard and Ritchie, 1971; DeWeer, 1975). Because the highest surface-to-volume ratios are found in nerve endings and dendrites, one would expect brain areas rich in these structures to have the greatest relative increase in glucose consumption accompanying stimulation. Recognition of this will be of importance in interpreting future studies using the autoradiographic deoxyglucose method.

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